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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/695,546	10/28/2003	Stephen Barnes	200123-2	7512

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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)
	10/695,546	BARNES ET AL.
	Examiner	Art Unit
	Stephen Kapushoc	1634

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 May 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-25* is/are pending in the application.

4a) Of the above claim(s) *22 and 23* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,4-11,14-21,24 and 25* is/are rejected.

7)☒ Claim(s) *2,3,12 and 13* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *28 October 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *6/25/04; 10/28/03*.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: _____.

DETAILED ACTION

Claims 1-25 are pending
Claims 1-21 and 24-25 are examined on the merits.

Election/Restrictions

1. Applicant's election without traverse of Group I, claims 1-21 and 24-25 in the reply filed on 05/10/2006 is acknowledged. Claims 22 and 23 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Specification

2. The disclosure is objected to because of the following informalities:

Page 11, paragraph [040] reads 'any or the purification methods' where 'any of the purification methods' may be intended. The Examiner has not checked the entire specification for typographical and grammatical errors, it is requested that Applicant corrects any such errors of which Applicant becomes aware.

Appropriate correction is required.

Claim Objections

3. Claims 1, 21, and 24 recite the phrase 'wherein the PM1 forward primer and PM1 reverse primer are nested within the AHAS1 forward and reverse primers' in step (d) of claim 1, step (i) of claim 21, and step (d) of claim 24. The objectionable phrase makes the nature of the PM1 forward and reverse primers unclear as to whether Applicant

intends for the PM1 primers to contain sequence identical to the AHAS1 primers, hybridize to overlapping binding sites, or hybridize to binding sites that are within the product created by amplification using the AHAS1 forward and reverse primers. The phrase may be made clearer if edited to read 'wherein the binding sites of the PM1 forward primer and PM1 reverse primer are nested within the amplified portion of the AHAS1 gene', if that is what applicant intends.

Claim Rejections - 35 USC § 112 2nd –Indefiniteness

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 4-6 and 14-19 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 4, 5, and 14-17 are unclear over the recitation of the phrase 'has a sequence as set forth in'. Claim language using the indefinite article 'a' thus refers to any portion of the claimed SEQ ID NO, including as little as a single nucleotide, and it is thus unclear as to what the structural limitations of the claimed nucleic acid sequences are. The claims made be made more clear if the unclear phrase is changed to read 'has the sequence as set forth in'.

Claim 6 is unclear over the limitation 'wherein step (c) includes incorporating a label into the amplified portion of the *AHAS1* gene'. Step (c) of claim 1 involves purification of the *AHAS1* reaction mixture. It is unclear how incorporating a label in this

step achieves detection of the PM1 mutation. The claim may be made more clear if edited to read 'wherein step (d) includes incorporating a label into the amplified portion of the *AHAS1* gene', which would be in agreement with the teachings of the specification (paragraph [035] on page 10) which teach labeling of the amplified portion of the *AHAS1* gene that contains the PM1 mutation during amplification with SEQ ID NOs: 11 and 12.

Claims 18 and 19 are unclear over the limitation 'wherein steps (c) and (d) include incorporating a label into the amplified portion of the *AHAS3* gene' in claim 18. Step (c) of claim 11 involves purification of the *AHAS1* reaction mixture, step (d) is amplification using a primer selective for the wild type allele of the PM2 region. It is unclear how incorporating a label in these steps achieves detection of the PM2 mutation. The claim may be made more clear if edited to read 'wherein step (d) and (e) include incorporating a label into the amplified portion of the *AHAS3* gene', which would be in agreement with the teachings of the specification (paragraph [044] on page 12) which teach labeling of the amplified portion of the *AHAS3* gene that contains the PM2 mutation during amplification with allele specific oligonucleotides.

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 1, 4-9, and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rutledge et al (1991, as cited in the IDS) in view of Sathasivan et al (1991), and Shi et al (1996).

Rutledge et al teaches the nucleic acid and deduced amino acid sequence of the *Brassica AHAS1* gene (Fig. 2A). The reference teaches that DNA was isolated from leaf nuclei, relevant to claim 1 step (a), and claim 24 step (a). The reference also teaches that imidazolinone herbicides act through inhibition of AHAS (p.39, left column, last paragraph), and further teaches that herbicide resistance in *Brassica* mutants results from two unlinked alleles, and that the effect of combining the alleles in a hybrid line is additive for imidazolinone resistance. The reference teaches that *AHAS1* is an imidazolinone resistance allele (p.39, left column, last paragraph), and concludes that the sequences of the *AHAS* genes provides the basic information essential for the analysis of *Brassica* mutants with resistance to herbicides that act on *AHAS* (p.39, right column, last paragraph).

Regarding claim 4, the sequence of the AHAS1 gene taught by Rutledge et al (Fig 2A) includes the sequence of the primer set forth in SEQ ID NO: 11 of the instant specification. Nucleotides 4364-4384 of the AHAS3 gene taught by Rutledge et al are identical to nucleotides 1-21 of SEQ ID NO: 11.

Regarding claim 5, the sequence of the AHAS1 gene taught by Rutledge et al (Fig 2A) includes the sequence of the primer set forth in SEQ ID NO: 12 of the instant specification. Nucleotides 4589-4609 of the AHAS3 gene taught by Rutledge et al are complementary to nucleotides 1-21 of SEQ ID NO: 12.

Rutledge et al does not teach the nature of the mutation in the AHAS1 gene (PM1) that confers resistance to imidazolinone.

Sathasivan et al teaches the analysis of an *A. thaliana* mutation in the acetolactate synthase gene (referred to within the reference as ALS, which is an art recognized synonym for AHAS). The reference teaches that the mutation provides the molecular basis for imidazolinone resistance in *A. thaliana* (p.1044 – Abstract). Sathasivan et al teaches the specific nature of the *A. thaliana* mutation responsible for herbicide resistance as a G to A single-point mutation at nucleotide 1958 of the coding sequence, which predicts a serine to asparagine substitution at amino acid 653 (p.1044, left column, last paragraph; Fig. 2; Table 1). The reference also provides an alignment indicating the conservation of the deduced amino acid residues in the acetolactate synthase gene near the mutation site conferring imidazolinone resistance (Fig. 3). Based on the teachings of Sathasivan et al (i.e. the sequence provided in Fig 2 and the alignment provided in Fig. 3), and the teaching of Rutledge et al (i.e.: the sequence of the AHAS1 gene together with the teaching that a mutation in AHAS1 provides imidazolinone resistance), it is evident that the *A. thaliana* G to A mutation taught by Sathasivan is equivalent to the PM1 mutation claimed in the instant application. The reference also teaches that similar mutations at corresponding nucleotide positions of other acetolactate synthase genes can confer imidazolinone resistance (p.1049, left column, last paragraph).

Sathasivan et al does not provide a method for the detection of the PM1 mutation comprising analysis of intramolecular interactions of an amplification product created by nested PCR.

Shi et al teaches a method for the analysis of mutations using single-strand conformational polymorphism (SSCP) analysis. Relevant to steps (b) and (d) of claim 1 and steps (b) and (d) of claim 24, the reference teaches that PCR products of target regions that were amplified using a first set of primers were subsequently reamplified using a second set of primers (p.272 – Nested PCR with exonuclease I selection; Table 1). The method of amplification taught by Shi et al comprises a first selective amplification of a gene region of interest using forward and reverse primers (relevant to step (b) of claims 1 and 24), followed by a step of removing remaining outer primers using exonuclease I (relevant to step (c) of claims 1 and 24), and a subsequent amplification of a portion of the gene having a mutation using forward and reverse primers nested within the primers used for the first amplification (relevant to step (d) of claims 1 and 24). The reference further teaches analysis of the amplified products using SSCP by denaturing the amplification product to produce single stranded polynucleotides that are allowed to adopt particular conformations and the detection of mutations based on mobility of the strands through a substrate (p.273 – SSCP analysis; p.271, right col., last paragraph; Fig. 1), relevant to steps (e) and (f) of claims 1 and 24.

Regarding claims 6 and 7, the reference teaches the incorporation of a radioactive label into the amplification product (p.273, left col., first full paragraph).

Regarding claim 8, the reference teaches the analysis of amplification products using a substrate that is polyacrylamide (p.273 – SSCP analysis).

Regarding claim 9, the reference teaches detection using electrophoresis (p.273 – SSCP analysis).

Thus it would have been prima facie obvious to one of skill in the art at the time the invention was made to have combined the information and methods provided in the cited references to have created the claimed invention of a method to assay imidazolinone resistance in a *Brassica* plant conferred by the PM1 mutation of the *AHAS1* gene. One would have been motivated to develop such an assay to efficiently determine the presence of herbicide resistance in a plant using molecular techniques. One would have had a reasonable expectation of success because the cited references teach both the general aspects of the properties responsible for imidazolinone resistance, as well as the specific molecular characteristics that confer imidazolinone herbicide resistance. Rutledge et al teaches that an allele of the *AHAS1* gene is responsible for imidazolinone resistance in a *B. napus* (p.39, right column, last paragraph). Rutledge et al further teaches the nucleic acid sequence and deduced amino acid sequence of the *B. napus AHAS1* gene (Fig. 2A). Sathasivan et al teaches the identification of a G to A (serine to asparagine) mutation in the *A. thaliana* ALS gene and provides an amino acid alignment indicating that this mutation is equivalent to the PM1 mutation of the instant application, and teaches that the mutation is applicable to *Brassica*. One would have been motivated to use the particular methods of Shi et al based on the assertion of Shi et al that the method is simple, effective, rapid, and

inexpensive (p.276, right col., lns.22-24) and provides improved final results in nucleic acid amplification based methods (p.274, left col., lns.12-13).

Regarding the specific primers recited in claims 4 and 5 (SEQ ID NO: 11 and 12), it would be obvious to use primers comprising or consisting of these sequences because these sequences are taught by Rutledge et al, and Sathasivan et al teaches the position of the PM1 mutation as nested between these nucleic acid sequences. Further, the method of Shi et al teaches using amplification primers that flank a region of interest for SSCP analysis. It would thus be obvious to use any primers (based on the sequences provided by Rutledge et al) flanking the position of the PM1 mutation (as taught by Sathasivan et al), including primers comprising or consisting of SEQ ID NO: 11 and 12, for analysis of the PM1 mutation using SSCP (as taught by Shi et al).

Regarding claim 24, Rutledge et al also teaches combining different *AHAS* mutants in a hybrid line. It would thus be obvious to select of plants identified as having the PM1 mutation for further breeding (relevant to step (g) of claim 24), to create plants with higher herbicide resistance (p.39, left col., last paragraph).

8. Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Rutledge et al (1991, as cited in the IDS) in view of Sathasivan et al (1991) and Shi et al (1996) as applied to claims 1, 4-9, and 24, and further in view of Hattori et al (1995, as cited in the IDS).

Rutledge et al in view of Sathasivan et al and Shi et al teaches a method for assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM1

mutation of the *AHAS1* gene comprising all of the limitations of claim 1, from which the rejected claim depends.

In addition to the sequence of the *AHAS1* gene, Rutledge et al teaches the nucleic acid and deduced amino acid sequence of the *Brassica AHAS3* gene (Fig. 2C). The reference also teaches that imidazolinone herbicides act through inhibition of *AHAS* (p.39, left column, last paragraph), and further teaches that herbicide resistance in *Brassica* mutants results from two unlinked alleles, and that the effect of combining the alleles in a hybrid line is additive for imidazolinone resistance. The reference teaches that in addition to *AHAS1*, *AHAS3* is an imidazolinone resistance allele (p.39, left column, last paragraph).

Rutledge et al does not teach the nature of the mutation in the AHAS3 gene (PM2) that confers resistance to imidazolinone.

Hattori et al teaches the analysis of the AHAS3 gene from imidazolinone-resistant mutant *Brassica* cells. The reference teaches that the AHAS3 gene from the mutant cells was cloned and sequenced, and the sequence of the gene from the mutant was compared to the wild-type AHAS3 sequence (p.420, right column, l.28). Hattori teaches the identification of a single basepair change (G to T) in AHAS3 that predicts a tryptophan to leucine amino acid change (p.421, left column, last paragraph), and provides a comparative alignment of deduced amino acid sequences in the region of the AHAS3 mutation responsible for herbicide resistance (p.421 Fig. 2). Based on the alignment provided in Fig. 2 of Hattori et al, and the sequence of the AHAS3 gene provided by Rutledge et al, it is evident that the G to T mutation taught by Hattori is

equivalent to the PM2 mutation claimed in the instant application. Hattori concludes that the identified mutation site in the AHAS3 gene is involved in the binding of imidazolinone herbicides, and teaches that the recovery of the same mutation in tobacco and *B. napus*. Further relevant to step (b) of claim 1 and step (b) of claim 25, Hattori et al teaches amplification of the *AHAS3* gene using a forward and reverse primer (p.420 – Isolation of *AHAS1* and *AHAS3* genes from herbicide resistant C20 line of *B. napus* callus).

It would have been prima facie obvious at the time the invention was made to have combined the methods of Rutledge et al in view of Sathasivan et al and Shi et al to include further detection of the PM2 mutation taught by Hattori et al. One would have been motivated to detect both mutations based on the teachings of Rutledge et al that combination of the two alleles in a single hybrid plant has an additive effect on imidazolinone resistance (p.39, left col., last paragraph), and the teachings of Hattori et al that the mutation taught by Hattori et al confers resistance to imidazolinone.

9. Claims 11, 14-20 and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rutledge et al (1991, as cited in the IDS) in view of Hattori et al (1995, as cited in the IDS), Liu et al (2001, US Patent 6,207,425), and Shi et al (1996).

Rutledge et al teaches the nucleic acid and deduced amino acid sequence of the *Brassica AHAS3* gene (Fig. 2C). The reference teaches that DNA was isolated from leaf nuclei, relevant to claim 11 step (a), and claim 25 step (a). The reference also teaches that imidazolinone herbicides act through inhibition of *AHAS* (p.39, left column,

last paragraph), and further teaches that herbicide resistance in *Brassica* mutants results from two unlinked alleles, and that the effect of combining the alleles in a hybrid line is additive for imidazolinone resistance. The reference teaches that *AHAS3* is an imidazolinone resistance allele (p.39, left column, last paragraph), and concludes that the sequences of the *AHAS* genes provides the basic information essential for the analysis of *Brassica* mutants with resistance to herbicides that act on *AHAS* (p.39, right column, last paragraph).

Regarding claim 14, the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) includes the sequence of the primer set forth in SEQ ID NO: 15 of the instant specification. Nucleotides 1730-1748 of the AHAS3 gene taught by Rutledge et al are identical to nucleotides 1-19 of SEQ ID NO: 15.

Regarding claim 15, the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) includes the sequence of the primer set forth in SEQ ID NO: 16 of the instant specification. Nucleotides 2117-2099 of the AHAS3 gene taught by Rutledge et al are complementary to nucleotides 1-19 of SEQ ID NO: 16.

Regarding claim 16, the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) includes the sequence of the primer set forth in SEQ ID NO: 17 of the instant specification. Nucleotides 1907-1924 of the AHAS3 gene taught by Rutledge et al are identical to nucleotides 1-18 of SEQ ID NO: 17.

Regarding claim 16, the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) includes the portion of the sequence of the primer set forth in SEQ ID NO: 18 of the instant specification that is not specific for the PM2 mutation. Nucleotides 1905-

1923 of the AHAS3 gene taught by Rutledge et al are identical to nucleotides 1-19 of SEQ ID NO: 18.

Rutledge et al does not teach the nature of the mutation in the AHAS3 gene (PM2) that confers resistance to imidazolinone.

Hattori et al teaches the analysis of the AHAS3 gene from imidazolinone-resistant mutant *Brassica* cells. The reference teaches that the AHAS3 gene from the mutant cells was cloned and sequenced, and the sequence of the gene from the mutant was compared to the wild-type AHAS3 sequence (p.420, right column, l.28). Hattori teaches the identification of a single basepair change (G to T) in AHAS3 that predicts a tryptophan to leucine amino acid change (p.421, left column, last paragraph), and provides a comparative alignment of deduced amino acid sequences in the region of the AHAS3 mutation responsible for herbicide resistance (p.421 Fig. 2). Based on the alignment provided in Fig. 2 of Hattori et al, and the sequence of the AHAS3 gene provided by Rutledge et al, it is evident that the G to T mutation taught by Hattori is equivalent to the PM2 mutation claimed in the instant application. Hattori concludes that the identified mutation site in the AHAS3 gene is involved in the binding of imidazolinone herbicides, and teaches that the recovery of the same mutation in tobacco and *B. napus*. Further relevant to step (b) of claim 1 and step (b) of claim 25, Hattori et al teaches amplification of the *AHAS3* gene using a forward and reverse primer (p.420 – Isolation of *AHAS1* and *AHAS3* genes from herbicide resistant C20 line of *B. napus* callus).

Hattori does not teach performing second and third amplification steps using region specific forward and reverse primers and primers specific for the wild type or PM2 mutation allele of the *AHAS3* gene.

Liu et al teaches a method for the bidirectional PCR amplification of specific alleles (Bi-PASA) for the detection of mutations (Figure 1A; col.4 lns. 30-51), relevant to steps (d) and (e) of claims 11 and 25. The reference teaches that amplification is carried out using region specific forward and reverse primers (identified as 'P' and 'Q' in the reference) which provide a positive control (col. 4 lns.53-54), as well as allele specific primers (identified as 'A' and 'B' in the reference) which are selective for the mutant and wildtype alleles of the analyzed gene (col.4 lns.54-57). The reference further teaches that the method can be performed using all four primers in one reaction, or as separate reactions using different combinations of useful primers (col.5 lns 31-36), and specifically teaches the analysis of reactions containing primers PQB or PQA (e.g.: Fig. 3A lanes 16 and 17, respectively). Relevant to claims 18 and 19, the reference teaches incorporation of a radioactive label into the amplified nucleic acid for purposes of analysis of the amplification products (col.3 lns.24-30; Fig 2; col.6 ln.15). Relevant to claim 20, the reference teaches analysis of amplification products by electrophoresis (col.6 lns.15-17).

Liu et al does not teach a method comprising the removal of primers from a previous gene amplification step, prior to a subsequent PCR amplification.

Shi et al teaches a method for the analysis of mutations nested PCR and removal of unincorporated primers. Relevant to steps (b) and (d) of claim 11 and steps

(b) and (d) of claim 25, the reference teaches that PCR products of target regions that were amplified using a first set of primers were subsequently reamplified using a second set of primers (p.272 – Nested PCR with exonuclease I selection; Table 1). The method of amplification taught by Shi et al comprises a first selective amplification of a gene region of interest using forward and reverse primers (relevant to step (b) of claims 11 and 25), followed by a step of removing remaining outer primers using exonuclease I (relevant to step (c) of claims 11 and 25), and a subsequent amplification of a portion of the gene having a mutation using forward and reverse primers nested within the primers used for the first amplification (relevant to step (d) of claims 1 and 25).

Thus it would have been prima facie obvious to one of skill in the art at the time the invention was made to have combined the information and methods provided in the cited references to have created the claimed invention of a method to assay imidazolinone resistance in a *Brassica* plant conferred by the PM2 mutation of the *AHAS3* gene. One would have been motivated to develop such an assay to efficiently determine the presence of herbicide resistance in a plant using molecular techniques. One would have had a reasonable expectation of success because the cited references teach both the general aspects of the properties responsible for imidazolinone resistance, as well as the specific molecular characteristics that confer imidazolinone herbicide resistance. Rutledge et al teaches that an allele of the *AHAS3* gene is responsible for imidazolinone resistance in a *B. napus* (p.39, right column, last paragraph). Rutledge et al further teaches the nucleic acid sequence and deduced amino acid sequence of the *B. napus* AHAS3 gene (Fig. 2C). Hattori et al teaches the

identification of a G to T (tryptophan to leucine) mutation in the *B. napus* AHAS3 gene responsible for imidazolinone resistance that is equivalent to the PM2 mutation of the instant application. One would have been motivated to use the particular techniques of Liu et al and Shi et al based on the teachings of Liu et al that the method is rapid and particularly useful for analysis of single-base change mutants (col. 2 lns.49-61) and the teachings of Shi et al that nested PCR methods using removal of primers prior to the second amplification are simple, effective, rapid, and inexpensive (p.276, right col., lns.22-24) and provide improved final results in nucleic acid amplification based methods (p.274, left col., lns.12-13). Combining the methods of Liu et al and Shi et al would create a method in which the gene of interest is first amplified using a first primer pair comprised of gene specific forward and reverse primers, followed by removal of primers, and then diagnostic amplifications using forward and reverse primers that are nested within the first primer pair and wild type or mutation specific primers.

Regarding the specific primers recited in claims 14, 15, and 17 (SEQ ID NO: 15, 16, and 18), it would be obvious to use primers comprising or consisting of these sequences because these sequences are taught by Rutledge et al; and Hattori et al teaches the position of the PM2 mutation as nested between the nucleic acid sequences of SEQ ID NO: 15 and 16, and the presence of the mutation creates a sequence comprising SEQ ID NO: 18. Further, the method of Liu et al teaches using amplification primers that flank a region of interest and an allele specific primer with a discriminating nucleotide at its 3'-end for mutation analysis. It would thus be obvious to use any primers (based on the sequences provided by Rutledge et al) flanking the

position of the PM2 mutation (as taught by Hattori et al), including primers comprising or consisting of SEQ ID NO: 14 and 15, as well as any primer with a PM2 specific nucleotide at its 3'-end, including a primer comprising or consisting of SEQ ID NO: 18, for analysis of the PM2 mutation using allele specific amplification.

Regarding claim 25, Rutledge et al also teaches combining different *AHAS* mutants in a hybrid line. It would thus be obvious to select of plants identified as having the PM2 mutation for further breeding (relevant to step (g) of claim 25), to create plants with higher herbicide resistance (p.39, left col., last paragraph).

10. Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Rutledge et al (1991, as cited in the IDS) in view of Hattori et al (1995, as cited in the IDS), Liu et al (2001, US Patent 6,207,425), and Shi et al (1996), as applied to claims 11, 14-20 and 25, and further in view of Sathasivan et al (1991).

Rutledge et al in view of Hattori et al, Liu et al, and Shi et al teaches a method for assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM2 mutation of the *AHAS3* gene comprising all of the limitations of claim 11, from which the rejected claim depends.

Shi et al additionally teaches analysis of the amplified products using SSCP by denaturing the amplification product to produce single stranded polynucleotides that are allowed to adopt particular conformations and the detection of mutations based on mobility of the strands through a substrate (p.273 – SSCP analysis; p.271, right col., last paragraph; Fig. 1), relevant to steps (j) and (k) of claim 21.

In addition to the sequence of the *AHAS3* gene, Rutledge et al teaches the nucleic acid and deduced amino acid sequence of the *Brassica AHAS1* gene (Fig. 2A). The reference also teaches that imidazolinone herbicides act through inhibition of *AHAS* (p.39, left column, last paragraph), and further teaches that herbicide resistance in *Brassica* mutants results from two unlinked alleles, and that the effect of combining the alleles in a hybrid line is additive for imidazolinone resistance. The reference teaches that in addition to *AHAS3*, *AHAS1* is an imidazolinone resistance allele (p.39, left column, last paragraph).

Rutledge et al does not teach the nature of the mutation in the *AHAS1* gene (PM1) that confers resistance to imidazolinone.

Sathasivan et al teaches the analysis of an *A. thaliana* mutation in the acetolactate synthase gene (referred to within the reference as ALS, which is an art recognized synonym for AHAS). The reference teaches that the mutation provides the molecular basis for imidazolinone resistance in *A. thaliana* (p.1044 – Abstract). Sathasivan et al teaches the specific nature of the *A. thaliana* mutation responsible for herbicide resistance as a G to A single-point mutation at nucleotide 1958 of the coding sequence, which predicts a serine to asparagine substitution at amino acid 653 (p.1044, left column, last paragraph; Fig. 2; Table 1). The reference also provides an alignment indicating the conservation of the deduced amino acid residues in the acetolactate synthase gene near the mutation site conferring imidazolinone resistance (Fig. 3). Based on the teachings of Sathasivan et al (i.e. the sequence provided in Fig 2 and the alignment provided in Fig. 3), and the teaching of Rutledge et al (i.e.: the sequence of

the AHAS1 gene together with the teaching that a mutation in AHAS1 provides imidazolinone resistance), it is evident that the *A. thaliana* G to A mutation taught by Sathasivan is equivalent to the PM1 mutation claimed in the instant application. The reference also teaches that similar mutations at corresponding nucleotide positions of other acetolactate synthase genes can confer imidazolinone resistance (p.1049, left column, last paragraph).

It would have been prima facie obvious at the time the invention was made to have combined the methods of Rutledge et al in view of Hattori et al, Liu et al, and Shi et al to have further included detection of the PM1 mutation taught by Sathasivan et al. One would have been motivated to detect both mutations based on the teachings of Rutledge et al that combination of the two alleles in a single hybrid plant has an additive effect on imidazolinone resistance (p.39, left col., last paragraph), and the assertion of Sathasivan et al that the mutation taught by Sathasivan et al confers imidazolinone resistance. One would have been motivated to use the SSCP method of Shi et al based on the assertion of Shi et al that the method is simple, effective, rapid, and inexpensive (p.276, right col., lns.22-24).

Requirement For Information Under 37 C.F.R. 1.105

11. Applicant and the assignee of this application are required under 37 CFR 1.105 to provide the following information that the examiner has determined is reasonably necessary to the examination of this application.

The May 2001 issue of the magazine Germination makes reference to an agreement between BASF and the Saskatchewan Wheat Pool in which the

Saskatchewan Wheat Pool develops herbicide-resistant canola under the Clearfield Production System. It is noted that the assignee of the invention of the instant application is BASF. The Examiner request the following information regarding the agreement: do the mentioned herbicide-resistant canola varieties contain the PM1 and PM2 mutations, and if so does the development of the new varieties include the nucleic acid based methods to detect the PM1 and PM2 mutations disclosed in the specification of the instant application.

12. This requirement is an attachment of the enclosed Office action. A complete reply to the enclosed Office action must include a complete reply to this requirement. The time period for reply to this requirement coincides with the time period for reply to the enclosed Office action.

Double Patenting

13. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

14. Claims 1, 4-11, 14-21, 24, and 25 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-5 of copending Application No. 10/695,089 in view of Shi et al (1996) and Liu et al (2001, US Patent 6,207,425.

The claims of the conflicting application are drawn to methods for determining the presence of the PM1 and PM2 mutations in genomic DNA isolated from *B. napus*. The rejected claims of the instant application are drawn to the analysis of the same mutations in the same genes of the same species of plants.

The claims of the copending application do not specifically recite the particular methods for mutation analysis (i.e. nested PCR SSCP, and nested PCR bi-directional PCR amplification of specific alleles). However, such methods were known in the art at the time the invention was made.

The art of Shi et al teaches a method for the analysis of mutations using single-strand conformational polymorphism (SSCP) analysis. The reference teaches that PCR products of target regions that were amplified using a first set of primers were subsequently reamplified using a second set of primers (p.272 – Nested PCR with exonuclease I selection; Table 1). The method of amplification taught by Shi et al comprises a first selective amplification of a gene region of interest using forward and reverse primers, followed by a step of removing remaining outer primers using exonuclease I, and a subsequent amplification of a portion of the gene having a

mutation using forward and reverse primers nested within the primers used for the first amplification. The reference further teaches analysis of the amplified products using SSCP by denaturing the amplification product to produce single stranded polynucleotides that are allowed to adopt particular conformations and the detection of mutations based on mobility of the strands through a substrate (p.273 – SSCP analysis; p.271, right col., last paragraph; Fig. 1).

The art of Liu et al teaches a method for the bidirectional PCR amplification of specific alleles (Bi-PASA) for the detection of mutations (Figure 1A; col.4 lns. 30-51). The reference teaches that amplification is carried out using region specific forward and reverse primers (identified as 'P' and 'Q' in the reference) which provide a positive control (col. 4 lns.53-54), as well as allele specific primers (identified as 'A' and 'B' in the reference) which are selective for the mutant and wildtype alleles of the analyzed gene (col.4 lns.54-57). The reference further teaches that the method can be performed using all four primers in one reaction, or as separate reactions using different combinations of useful primers (col.5 lns 31-36), and specifically teaches the analysis of reactions containing primers PQB or PQA (e.g.: Fig. 3A lanes 16 and 17, respectively). The reference teaches incorporation of a radioactive label into the amplified nucleic acid for purposes of analysis of the amplification products (col.3 lns.24-30; Fig 2; col.6 ln.15), and analysis of amplification products by electrophoresis (col.6 lns.15-17).

It would be prima facie obvious to use the methods of Shi et al and Liu et al to perfrm the mutation analysis methods claimed by 10/695,089. Using the methods of Shi et al and Liu et al to analyze the PM1 and PM2 mutations of *Brassica* would result in

the methods claimed in the instant application. Furthermore, such methods would include using the specific primers recited in claims 4, 5, 14, 15, and 17 (i.e. SEQ ID NO: 11, 12, 15, 16, and 18) as Shi et al and Liu et al teach using primers flanking the analyzed site, as well as allele specific primers with an allele specific nucleotide at the 3'-end.

This is a provisional obviousness-type double patenting rejection.

Conclusion - Claim Objections

15. Claims 1,4-11,14-21,24 and 25 are not allowable, the claims are not free of the prior art

16. Claims 2, 3, 12 and 13 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

17. Though the sequences of the *AHAS1* and *AHAS3* genes are disclosed in the prior art of Rutledge et al (1991, as cited in the IDS), the primers required by the objected to claims (i.e.: claim 2 requires SEQ ID NO: 9; claim 3 requires SEQ ID NO: 10; claim 12 requires SEQ ID NO: 13; claim 13 requires SEQ ID NO: 14) are comprised of sequences that deviate from the gene sequences disclosed by Rutledge et al. And while introduction of intentional mismatches within primers for the purposes of PCR amplification of different genes with similar sequences was known in the prior art (see for example Hung et al (1999)), there is no motivation in the prior art to modify

the sequences provide by Rutledge et al so as to arrive at the sequences claimed by the objected to claims.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephen Kapushoc whose telephone number is 571-272-3312. The examiner can normally be reached on Monday through Friday, from 8am until 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached at 571-272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Stephen Kapushoc
Art Unit 1634

JULIET C. SWITZER
PRIMARY EXAMINER